Original Article

Upregulation of fatty acid synthesis and the suppression of hepatic triglyceride lipase as a direct cause of hereditary postprandial hypertriglyceridemia in rabbits

Naoki Fukuda,[1] Tsunekata Ito,[2] Kazuo Ohwada[2,3] and Junichi Fujii[1,*]

[1]Department of Biochemistry and Molecular Biology, Graduate School of Medical Science, [2]Laboratory Animal Center, Yamagata University Faculty of Medicine and [3]Department of Comparative Medicine and Advanced Laboratory Animal Technology, Graduate School of Medical Science, Yamagata University, 2-2-2 Iidanishi, Yamagata 990-9585, Japan

(Received 19 February, 2013; Accepted 16 June, 2013; Published online 31 August, 2013)

Rabbits with hereditary postprandial hypertriglyceridemia exhibit central obesity and are regarded as a reliable model for metabolic syndrome. This study was performed to gain insight into the affected process of lipid metabolism and into the causative genes of the postprandial hypertriglyceridemia rabbits. Eleven genes that play key roles in lipid metabolism were selected, their mRNA levels were assessed by quantitative PCR, and their expressions were compared among postprandial hypertriglyceridemia rabbits using Japanese white rabbits as the control. Two genes appeared to be in causal connection with postprandial hypertriglyceridemia, and these were regarded as likely candidates for the pathogenesis. One was the fatty acid synthase gene, which had an expression constitutively higher in postprandial hypertriglyceridemia rabbits than in Japanese white rabbits during the fasting state and reached quite high levels after feeding. The other was the gene for hepatic triglyceride lipase with an expression that was approximately one order lower than that found in the Japanese white rabbits. The low plasma hepatic triglyceride lipase activities were consistent with the low levels of the transcript in the livers of the postprandial hypertriglyceridemia rabbits. Thus, elevated fatty acid synthesis and defected lipid hydrolysis together would cause the postprandial hypertriglyceridemia in postprandial hypertriglyceridemia rabbits.

Key Words: **postprandial hypertriglyceridemia, metabolic syndrome, hepatic triglyceride lipase, fatty acid synthase**

Obesity caused by overeating and immobilization is an underlying mechanism for cerebral vascular disease, cardiovascular disease, and malignant neoplasms that are major causes of death in developed countries.(1) U.S. statistics show that metabolic syndrome affects ~1 in 4 adults in that country, and that the incidence is dramatically increasing as the population grows.(2) The diagnostic criteria of metabolic syndrome are based on abnormalities in the abdominal circuit, in serum lipids, in blood pressure, and in glucose levels under fasting conditions.(3)

Although mice are popular animal models to elucidate the molecular mechanisms of diseases and to develop medicines due to the established technology in genetic modification, there are major differences between mice and humans from physiological and biochemical viewpoints. In this regard, rabbits are a preferable animal model, particularly with regard to lipid metabolism and the cardiovascular system.(4) Indeed, the Watanabe heritable hyperlipidemic (WHHL) rabbit has greatly contributed to our understanding of hypercholesterolemia and cardiovascular diseases.(5)

We have segregated hereditary postprandial hypertriglyceridemic (PHT) rabbits from the WHHL rabbit and established them as a model animal for metabolic syndrome.(6) Although the WHHL rabbit possesses 4-amino acid deletion in the cysteine-rich ligand-binding domain in the LDL receptor gene (LDLR) and shows severe hypercholesterolemia and atherosclerosis,(7) the PHT rabbit has a normal LDLR. The most prominent feature of PHT rabbits is postprandial hypertriglyceridemia. Visceral fat accumulation, hyperinsulinemia, and mild hypertension are observed in PHT rabbits.(6) Histological characterization of the PHT rabbit tissues reveals aortic intimal thickening and hepatic fatty degeneration.(8) Elevated secretion of VLDL from liver and temporary delaying of triglyceride degradation appears to be a direct cause of the postprandial hypertriglyceridemia.(9)

The PHT rabbit is thus regarded as an ideal model for studying the pathogenesis of human metabolic syndrome. The molecular mechanism of the pathogenesis, however, is not clearly understood, and the genetic basis for the syndrome is entirely unknown. In the present study, we sought to gain an understanding of the underlying mechanism for the syndrome in PHT rabbits by focusing on the expression of genes involved in the processes of lipid synthesis in the liver, degradation, and uptake by peripheral tissues. We found elevated expressions of the fatty acid synthase (FAS) gene and a decrease in the expression of the hepatic triglyceride lipase (HTGL) gene as well as lower HTGL activity compared to normal rabbits, which together would lead to pathogenic phenotypes in the PHT rabbit.

Materials and Methods

Animals. Male PHT rabbits, from Yamagata University as described previously,(6) and JW rabbits (Shiraishi Laboratory Animals, Tokyo, Japan) (10 rabbits from each group), all 11–18 months of age, were housed in an animal room maintained at 22 ± 2°C with humidity that ranged between 40 and 60% and a lighting period from 6:00 to 18:00 in the Laboratory Animal Center of Yamagata University School of Medicine. The rabbits were fed a standard diet (120 g per rabbit per day; Labo R-Grower, Nihonnosan, Yokohama, Japan) at noon daily. The nutritional composition and energy values of the feed were as follows: moisture, 7.4%; crude protein, 17.1%; crude fat, 5.4%; crude fiber,

*To whom correspondence should be addressed.
E-mail: jfujii@med.id.yamagata-u.ac.jp

doi: 10.3164/jcbn.13-21

17.1%; crude ash, 9.6%; crude nitrogen-free extract, 43.5%; and, metabolizable energy, 2,087 kcal/kg. Water was supplied ad libitum by an automatic watering system. Animal experiments were performed in accordance with the Declaration of Helsinki under protocols approved by the Animal Research Committee of Yamagata University.

Measurement of serum triglycerides and cholesterol. Blood samples (1 ml each) were collected from the auricular artery and were maintained at room temperature for 30 min. The samples then were centrifuged (3,000 rpm, 15 min, 4°C) to obtain supernatants, which were subjected to measurements of serum triglyceride (TG) and total cholesterol (T-CHO) levels. These parameters were determined using a Model 680 micro plate reader (BIO-RAD) through absorbance determination using Cholesterol and Triglyceride E-tests (Wako Pure Chemical Industries, Osaka, Japan).

Extraction of total RNA from tissues. Tissues samples were dissected from both the PHT ($n = 10$) and JW rabbits ($n = 10$) before (18–24 h fasting) and after 15–18 h continuous feeding of a 120 g normal diet. The rabbits were anesthetized with pentobarbital sodium (30 mg/kg, intravenously) followed by exsanguination. After dissection, brain, heart, artery, liver, kidney, adrenal, mesenteric adipose tissue, and muscle were immediately frozen in liquid nitrogen and stored at –80°C until RNA extraction. Total RNA was extracted using the RNeasy Lipid Tissue Mini Kit (QIAGEN, Tokyo, Japan). Concentrations of total RNAs were determined by spectrophotometer (E-spect, Malcom, Tokyo, Japan).

RT-PCR and real-time RT-PCR. The cDNA was synthesized from 1 μg of total RNA using a ReverTra Ace qPCR RT Kit (Toyobo, Osaka, Japan) and was subjected to PCR amplification using a PCR thermal cycler (ASTEC, model PC707, Japan). Primer sequences were designed based on the published data on rabbit genes and were used for RT-PCR and real-time RT-PCR. In case no proper primers were found in the literature, they were designed by using Primer3[10] or Primer-BLAST (NCBI, GenBank, BLAST). All PCR primers were designed to span at least one intron to distinguish PCR products generated from cDNA versus genomic DNA. These primer sequences together with sizes of the amplified fragments are shown in supplemental Table 1*. The reaction mixture contained 0.1 μl of KOD Dash, 1.0 μl of 10 × PCR buffer, 1.0 μl of forward primer, 1 μl of reverse primer, 1.0 μl of cDNA template, and 4.9 μl of RNase- and DNase-free water (Toyobo). Thirty cycles of denaturation (98°C for 10 s), annealing (55°C for 2 s), and extension (74°C for 30 s) were carried out in the thermal cycler. The PCR products were separated via 1.5% agarose (Ultra pure agarose, Invitrogen) gel electrophoresis and visualized by staining with ethidium bromide under UV light illumination. The glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene transcript was used as a reference for RNA integrity and assay conditions. The quantitative real-time RT-PCR was carried out using an Applied Biosystems 7500 Fast Sequence Detection System, and was performed in duplicate using a SYBR Green Master Mix (Applied Biosystems, Tokyo, Japan) according to the manufacturer's protocol. Rabbit 18-S rRNA was amplified as an endogenous control to normalize input RNA. The amplification conditions included 10 min at 95°C followed by 40 cycles of 5 s at 95°C and 20 s at 60°C. The melting curve protocol was performed for each primer set to confirm specificity.

Measurement of serum hepatic triglyceride lipase (HTGL) activity. Plasma HTGL activities were measured in male PHT rabbits ($n = 12$) and in JW rabbits ($n = 11–12$), all 4–6 months of age. After a 24-h fast, 500 U/kg heparin sodium (Novo-Heparin Injection 1000, Mochida Pharmaceutical Co. Ltd., Tokyo, Japan) was injected into the marginal ear vein of each rabbit. One ml blood was removed and added to the 4 ml-vacuum lithium heparin tubes (Benoject II, Terumo, Tokyo, Japan) 10 min after the heparin injection. Blood was centrifuged at 3,500 rpm and 4°C for 10 min, and plasma was collected and frozen in storage at –80°C until measurement. According to the method described by Coenen *et al.*[11], the HTGL activity was analyzed via a Hepatic Lipase Continuous Fluorometric Lipase Test from Progen (Heidelberg, Germany). The fluorescence of the HTGL reaction was measured in a Varioskan Flash microplate reader (Thermo Scientific, Tokyo, Japan) with an excitation wavelength at 342 nm and an emission wavelength at 452 nm in 1-min intervals over a 10-min period at 37°C. The slopes of the samples were compared to the slope of a standard that ranged from 1.4 to 22.5 pmol/ml.

Sequence analyses of HTGL cDNA and the promoter region of the HTGL gene. The total RNAs extracted from the livers of the PHT and JW rabbits were used to synthesize the cDNA. The nucleotide sequences of the exonic regions of HTGL were sequenced. Genomic DNA that was extracted from the kidneys using the SDS-proteinase K method was used to analyze the promoter region of HTGL, and spanned from the first exon to about 2,000 bp upstream. Three of each of the male PHT and JW rabbits were subjected to the sequence determination. The set of primers used were designed by the above-mentioned method based on the sequence data provided by GenBank (Accession No. AF041202) and Ensembl (LIPC_RABIT; No.17 chromosome 13970783–13972783).

Statistical analysis. Data are presented as the mean values + the standard error of the means (SEM). Statistical data were analyzed using SAS-JMP software (SAS Institute, Cary, NC). The significance of the differences between PHT and JW rabbits was evaluated using an F-test, followed by a Student's *t* test.

Results

Serum TG and total cholesterol (T-CHO) levels in PHT and JW rabbits. Pre- and post-prandial plasma TG and T-CHO levels were measured in the PHT and JW rabbits (Fig. 1). During the fasting period, TG levels were constitutively higher in PHT rabbits than in JW rabbits. Although feeding elevated plasma TG levels both in PHT rabbits and in JW rabbits, postprandial TG levels were 30 times higher in PHT rabbits than in JW rabbits. While T-CHO levels were not changed in the JW rabbits after feeding, a postprandial increase in T-CHO was observed in the PHT rabbits.

Expression of CD36 and NPC1L1 involved in lipid absorption in intestine. Postprandial hypertriglyceridemia can be caused by malfunction of several processes in lipid metabolism. We examined expression of CD36 and NPC1L1, which play essential roles in facilitating uptake of fatty acid and cholesterol, respectively,[12] in intestines of JW and PHT rabbits and their changes before and after meal. Analyses by quantitative PCR showed that expression of these genes were slightly higher in PHT rabbits than JW rabbits although they were not significantly different (Fig. 2).

Expression of genes related to lipid metabolism in various tissues. One noted difference from working with mice is that information on the DNA sequences of genes is limited, so a DNA microarray is not applicable for a study using rabbits. To better understand the metabolic changes controlled by gene expression, we selected and examined genes that play critical roles in the processes of lipid metabolism: fatty acid synthase (FAS), sterol regulatory element binding protein1 (SREBP-1), and SREBP-2 in lipid synthesis; microsomal triglyceride transfer protein (MTP) and cholesterol ester transfer protein (CETP) for lipid transfer to lipoproteins; lipoprotein lipase (LPL) and HTGL for lipid hydrolysis; and, LDLR and VLDL receptor (VLDLR) for lipoprotein uptake by cells. Total RNAs were extracted from various tissues including liver, heart, and mesenteric adipose tissue for both JW and PHT rabbits and these were subjected to RT-PCR followed by separation on agarose gels (Fig. 3). Primer pairs for each gene that amplified the DNA bands with expected sizes

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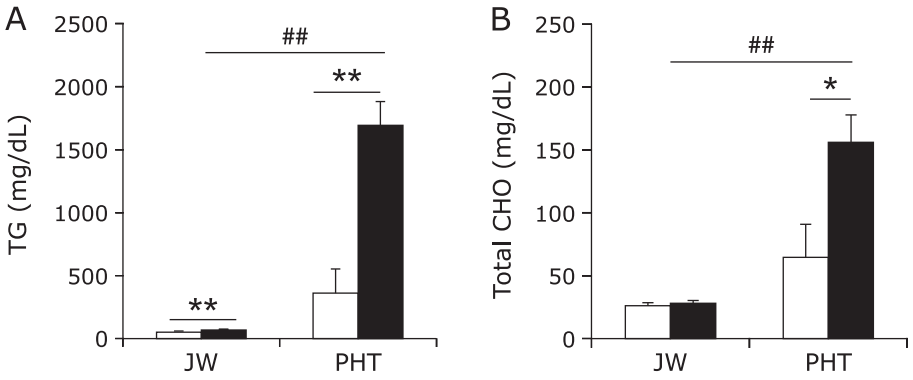

Fig. 1. Pre- and post-prandial plasma TG and T-CHO levels in PHT and JW rabbits. Levels of plasma TG (A) and T-CHO (B) were measured before (open box) or after (filled box) feeding. $n = 10$. $^{*}p<0.05$ and $^{**}p<0.01$ exhibited a significant difference between before and after feeding. $^{\#\#}p<0.01$ exhibited a significant difference between JW and PHT rabbits (Student's t test).

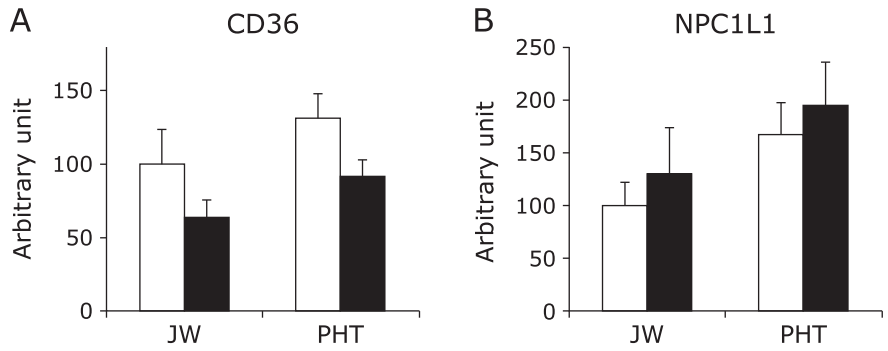

Fig. 2. Expression of CD36 and NPC1L1 involved in lipid absorption in the intestine. Total RNAs were extracted from the intestines of pre- (open box) and post-prandial (filled box) JW and PHT rabbits and were subjected to real-time RT-PCR. The expressions of CD36 (A) and NPC1L1 (B) were examined. $n = 5$–6. Data are expressed as the means ± SEM. No significant difference was found by Student's t test.

were used for the following quantitative PCR analyses. Very low expression of LPL was observed in aorta of the PHT rabbit compared to the JW rabbit. Because blood vessels are kept exposing to hyperlipidemic condition in the PHT rabbit, the gene expression may be dysfunctioned as reported.[8] Contribution by aorta in the lipid consumption is small due to its size, so we have not performed further analysis.

Expression of genes involved in lipid synthesis and transport in the liver. The liver regulates the synthesis of lipids through the control of plasma lipoproteins and their degradation. Therefore, we examined the expressions of 7 genes related to these activities in the liver (Fig. 4). Palmitic acid is primarily synthesized by the fatty acid synthase complex that is translated from a single gene FAS. Expression of FAS in the liver was originally high in the PHT rabbits, and feeding induced the expression of FAS in both JW and PHT rabbits. Although the mRNA levels after feeding were higher in PHT rabbits than in JW rabbits, the rates of increases in the FAS mRNA expression were similar. The expression of SREBP-1, which is a transcriptional regulator that is essential for FAS expression, was also originally higher in the PHT rabbits than in the JW rabbits, but was induced to similar levels in both JW and PHT rabbits. To the contrary, no difference was observed in the levels of SREBP-2 between JW and PHT rabbits. While the adrenal gland showed high levels of SREBP-2 mRNA, dietary conditions did not affect the expression (data not shown).

Then we examined two genes that are involved in apolipoprotein formation in the liver: MTP and CETP. The expressions of both MTP and CETP were the same for both PHT and JW rabbits and were not affected by nutritional conditions. However, we found a marked difference in the expression of HTGL mRNA between PHT and JW rabbits. The expression of the HTGL mRNA in the liver of PHT rabbits was less than 10% of that in JW rabbits, and was not altered after feeding. Regarding the genes involved in lipoprotein uptake by cells, we examined LDLR in the liver and found higher induction in PHT rabbits than in JW rabbits after feeding.

Expression of genes involved in lipolysis and lipoprotein uptake by the heart and adipose tissue. We then examined the genes involved in lipolysis and plasma lipoprotein uptake by tissues. Since the heart and the skeletal muscles utilize lipids as a major energy source and adipose tissue accumulates lipids, we examined the expression of lipoprotein lipase (LPL) and VLDLR in these tissues (Fig. 5). Under fasting conditions, levels of the LPL mRNA in the heart of PHT rabbits were slightly higher than those of JW rabbits, but these levels decreased after feeding. The sustained high levels of TG appear to cause down regulation of the LPL gene in the heart of the PHT rabbit, although further study is required to clarify this issue. Neither genetic background nor nutritional conditions affected the LPL mRNA level in femoral muscle and adipose tissues. Levels of VLDLR mRNA in these tissues were not affected by either lineage or nutritional conditions.

Lower plasma HTGL activity in PHT rabbits than in JW rabbits. Because levels of HTGL mRNA were low in the livers of the PHT rabbits, we attempted to confirm its contribution to plasma TG levels by measuring the HTGL activity in plasma. The rabbits were first administered heparin, which releases all the

doi: 10.3164/jcbn.13-21

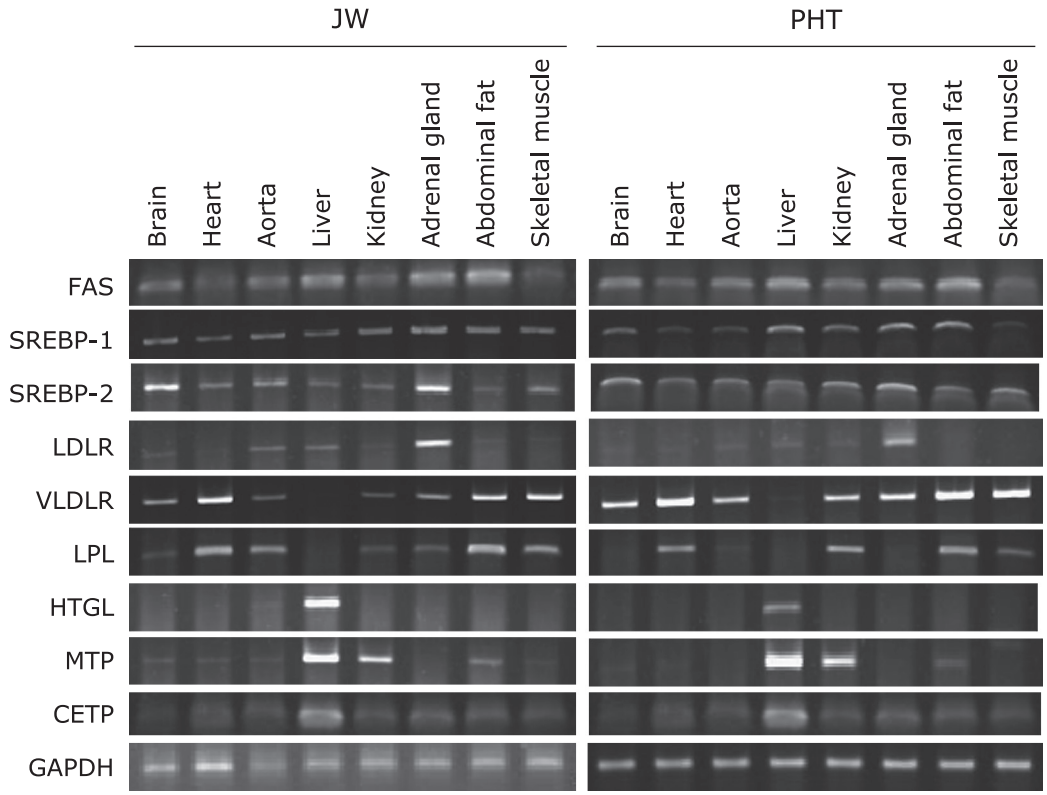

Fig. 3. RT-PCR analyses of the expression of 9 genes involved in lipid metabolism in various organs of PHT and JW rabbits. Total RNAs were extracted from various organs of PHT and JW rabbits and were subjected to RT-PCR. The resultant PCR products were separated on 1.5% agarose gel electrophoresis and visualized by staining with ethidium bromide using GAPDH as an internal standard.

HTGL that is bound to blood vessel walls into the plasma, and then blood was collected from them. The HTGL activity was significantly lower in the serum of PHT rabbits compared with that in JW rabbits in both the pre- and post-prandial states (Fig. 6) and was consistent with the mRNA levels.

Sequence analysis of the entire HTGL cDNA and promoter regions of the HTGL gene. To rationalize the low HTGL expression in the livers of the PHT rabbits, we performed sequence analysis of the HTGL gene. Fig. 7 shows the strategy used to determine the entire HTGL cDNA and the 5' promoter region and the 3' untranslated region of the HTGL gene for PHT and JW rabbits. A comparison of the cDNA sequences between PHT and JW rabbits revealed a single nucleotide polymorphism, C to T, in the exon 8 of the rabbit HTGL gene, but it was a silent mutation encoding Ser204. We also sequenced the promoter/regulatory region at approximately 2,000 bp because plural transcription factors bind to the ~1557 bp region in the human HTGL gene.[13,14] As a result, however, we found no difference in the sequences of the gene between the strains. Thus, it is unlikely that a mutation of the HTGL gene causes the suppressed expression in the PHT rabbit.

Discussion

As shown in Fig. 1, a typical phenotypic characteristic of PHT rabbits is a postprandial increase in plasma TG, which is seen neither in the JW nor in any other type of rabbit. Expression of CD36 and NPC1L1, which are two essential genes in intestinal lipid absorption,[12] was slightly elevated in the intestine of the PHT rabbits (Fig. 2) and was consistent with observation by Kawai *et al.* (manuscript in preparation) who found higher lipid absorption in PHT rabbits than JW rabbits. However, PHT rabbits were light in body weight compared to JW rabbits (Supplemental Fig. 1*), indicating that the elevated lipid absorption by intestine was not major cause for postprandial hypertriglyceridemia.

Obesity and food intake activate SREBP-1, which then promotes fatty acid synthesis by elevating the transcription of the genes, such as FAS, that are involved in fatty acid synthesis.[15] We found a constitutively high expression of both FAS and SREBP-1 under fasting conditions for PHT rabbits (Fig. 4), which suggests that FAS expression is upregulated by the elevated SREBP-1 at the transcriptional stage in PHT rabbits. Although feeding induced expression of the SREBP-1 mRNA in both PHT and JW rabbits to a similar extent, more SREBP-1 protein would exist in PHT rabbits than in JW rabbits because of its high constitutive levels of the transcript in PHT rabbits. A similar dissociation between SREBP-1 mRNA and FAS mRNA has been reported in rat adipocyte cells.[16] Insulin is known to induce the FAS mRNA expression via SREBP-1 activation,[17] and high levels of insulin are actually present in PHT rabbits compared to JW rabbits.[6] Thus, hyperinsulinemia would be at least partly responsible for the high levels of the SREBP-1 mRNA, and consequently the FAS mRNA.

T-CHO levels were similar for both PHT and JW rabbits under fasting conditions, but feeding increased the T-CHO levels only in PHT rabbits (Fig. 1). It is commonly observed that levels of T-CHO do not change after meal among mammals including human. So the observation in JW rabbits was normal response. SREBP-2 is involved mainly in cholesterologenesis.[15] The levels of SREBP-2 mRNA were increased to a similar extent for both PHT and JW rabbits after feeding. This suggests that a defect in the consumption of plasma cholesterol, rather than cholesterologenesis, was the cause in the PHT rabbits. LDLR mRNA in the liver was higher in PHT than in JW rabbits in the postprandial

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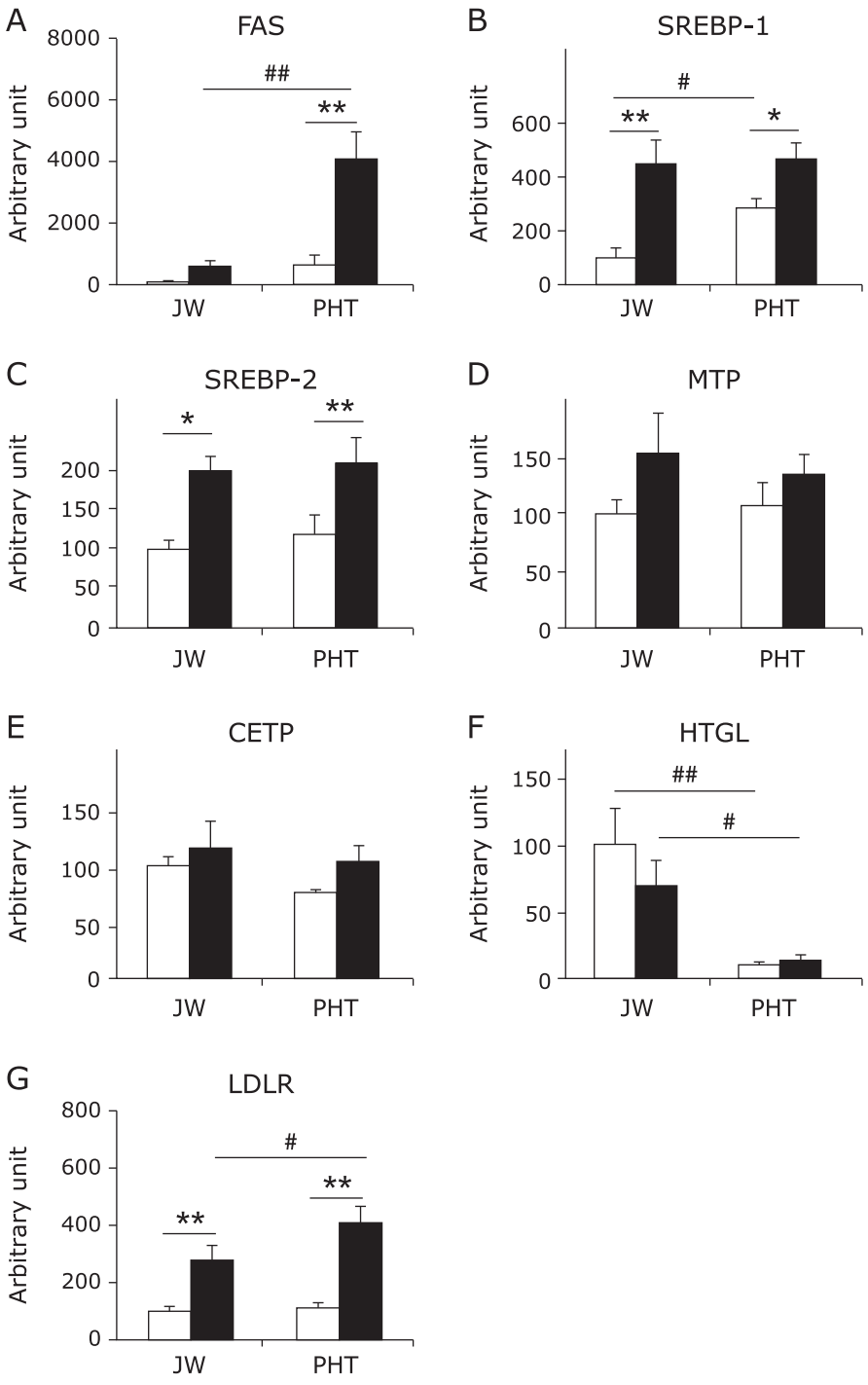

Fig. 4. Expression of 7 genes involved in lipid metabolism in the liver. Total RNAs were extracted from the livers of pre- (open box) and post-prandial (filled box) JW and PHT rabbits and were subjected to real-time RT-PCR. The expressions of 7 genes were examined (A–G). $n = 5$. Data are expressed as the means ± SEM. *$p<0.05$ and **$p<0.01$ exhibits for pre-prandial vs post-prandial, $^{\#}p<0.05$ and $^{\#\#}p<0.01$ exhibited significant differences between JW and PHT rabbits (Student's *t* test).

state, hence, the LDL uptake by the liver was not defected. Despite lower cholesterol contents in VLDL compared to LDL, the PHT rabbits contained extraordinarily high levels of VLDL,[9] suggesting that the VLDL-derived cholesterol would partly contribute to the high T-CHO values. Thus, the increase in the T-CHO can be explained by defected apolipoprotein clearance without increase in the CHO biosynthesis.

Regarding the expressions of MTP and CETP in the liver, no differences were observed by either dietary conditions or genetic backgrounds of the rabbits, indicating that the process to transport TGs or exchange cholesterol was not affected. The mRNA for LPL, which primarily hydrolyzes the TGs of VLDL and chylomicron (CM) and converts them to remnant lipoproteins,[18] was decreased after feeding in the PHT rabbits but was not different in the adipose tissues of the groups (Fig. 5). Thus, it is unlikely that these genes are involved in the pathogenesis of PHT rabbits.

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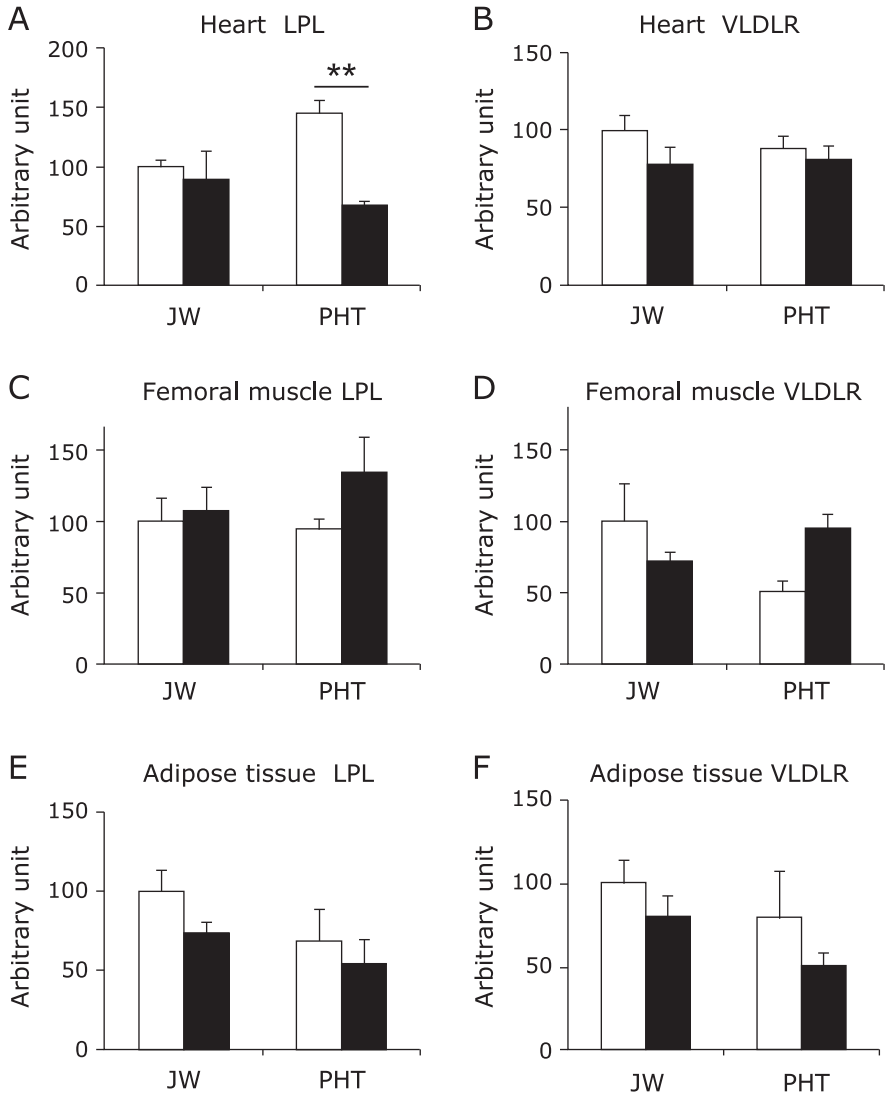

Fig. 5. Expression of LPL and VLDLR mRNA in the heart and adiopose tissues. Total RNAs were extracted from the heart (A and B) and adipose tissue (C and D) of pre- (open box) and post-prandial JW and PHT rabbits (filled box) and were subjected to real-time RT-PCR. The expressions of LPL and VLDLR were examined. $n = 5$. Data are expressed as the means ± SEM. *$p<0.05$ and **$p<0.01$ exhibited significant differences between the pre-prandial and post-prandial state. $^{\#}p<0.05$ and $^{\#\#}p<0.01$ exhibited significant differences between JW and PHT rabbits (Student's t test).

HTGL, which is synthesized in the hepatic cells and secreted to blood, hydrolyzes the TGs in lipoproteins and leads to TG incorporation into tissues.[13] The amounts of HTGL mRNA were markedly lower in the livers of PHT rabbits than in those of JW rabbits under fasting conditions and were not altered after feeding. HTGL activities that were lower in the PHT rabbits than in the JW rabbits were consistent with the defected gene expression (Fig. 6). As transgenic rabbits that express human HTGL cDNA in the liver show decreased levels of TGs and T-CHO in plasma,[19] it is reasonable to presume that the downregulation of the HTGL mRNA that was observed in PHT rabbits (Fig. 4) conversely contributed to the increase in the TG levels of PHT rabbits in the postprandial state. Thus, these results suggest that, together with elevated fatty acid synthesis, the suppressed expression of HTGL in PHT rabbits is one of the primary causes of postprandial hypertriglyceridemia.[20]

LDLR is involved in the cellular uptake of LDL, VLDL and VLDL remnant and hence contributes to lower levels of plasma cholesterol.[21] LDLR mRNA expression levels in the liver were increased in both strains after feeding. This tendency was similar to the mRNA expression of SREBP-2 and consistent with the role of SREBP-2 in regulating the expression of the LDLR.[22] In a similar manner, VLDLR is involved in the cellular uptake of chylomicron, VLDL and VLDL remnant and hence contributes to lower levels of plasma TGs.[23] Expression of VLDLR was high in heart and adipose tissues but not affected by either the dietary conditions or the genetic background of the rabbits (Fig. 5), indicating that VLDLR is not responsible for the pathogenesis of PHT rabbits.

Based on these results, the postprandial hypertriglyceridemia in PHT rabbits appears to be caused by the combined metabolic defects of lipid metabolism—upregulated fatty acid synthesis characterized as sustained FAS expression and defected TG consumption due to the suppressed HTGL expression. The sustained SREBP-1 expression would mediate augmented FAS expression, and hyperinsulinemia is an underlying mechanism.[6] To elucidate the mechanism for the suppressed HTGL expression, we performed sequence analyses of the HTGL gene and compared the nucleotide sequences between PHT and JW rabbits. We found a silent mutation with one base replacement at the 5th exon

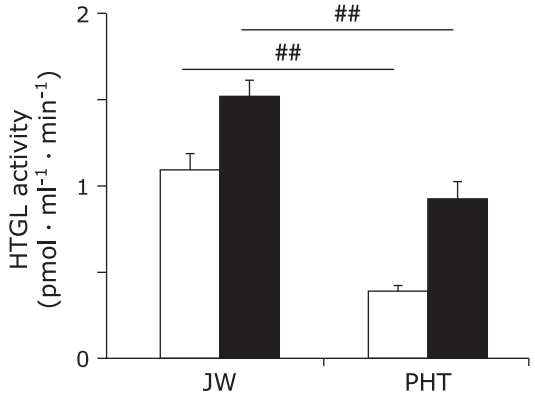

Fig. 6. Plasma HTGL activities in PHT and JW rabbits before and after feeding. Rabbits were either fasted for 48 h (open box) or fed for 18 h (filled box) (each group: n = 11–12). The marginal ear vein was used to administer 500 units/kg of heparin to each rabbit. After 10 min, 1-ml of blood was drawn, and HTGL activities were measured using a kit. Data are expressed as the mean ± SEM. $^{\#\#}p<0.01$ exhibited significant differences between JW and PHT rabbits (Student's t test).

(Fig. 7). Even if the change is not accompanied by amino acid replacement, there remains the possibility that the mutation may cause suppressed HTGL mRNA levels by decreasing the mRNA stability.[24] There was also the possibility that transcriptional activation of the gene was defected by a mutation in its 5'-upstream regulatory region. We performed sequence analysis of the 5' upstream promote/regulatory region of the HTGL gene up to about 2,000 bp covering essential DNA elements for the human HTGL gene,[25] but we failed to detect any difference between JW and PHT rabbits. Because we did not analyze intronic sequences, which may have mutations in the essential elements for gene expressions such as micro RNA, there remains the possibility of a defect in such elements of the HTGL gene in PHT rabbits. Since information on the regulatory mechanism of the HTGL gene is limited, further analyses are required to elucidate this. Because decreased HTGL activity is one of causes for postprandial hypertriglyceridemia in the PHT rabbit, an increase in the activity due to either direct activation of the HTGL enzyme or by induction of the HTGL gene would be a potential therapeutics to control postprandial hypertriglyceridemia and may be applicable for treatment of metabolic syndrome.

We concluded that the postprandial hypertriglyceridemia observed in PHT rabbits is caused mainly by augmented production and defected clearance of TGs from plasma. Constitutively high expression of FAS and suppressed expression of HTGL are involved in the overproduction and in the decreased clearance of plasma TGs, respectively. Hyperinsulinemia-triggered elevation of the SREBP-1 expression would underlie the upregulation of FAS expression. On the other hand, it is unclear what actually causes the suppressed expression of the HTGL gene. A clear understanding of these mechanisms may provide clues to overcoming the metabolic syndrome, and in this regard PHT rabbits are useful animal models for such a purpose.

Acknowledgments

We would like to thank Drs. X Zhang and Y Iuchi (Department of Biochemistry and Molecular Biology, Graduate School of Medical Science, Yamagata University), who instructed us the experimental techniques in this study. We also thank all the staff of the Laboratory Animal Center (Yamagata University Faculty of Medicine), who instructed and helped with the breeding and selection of animals.

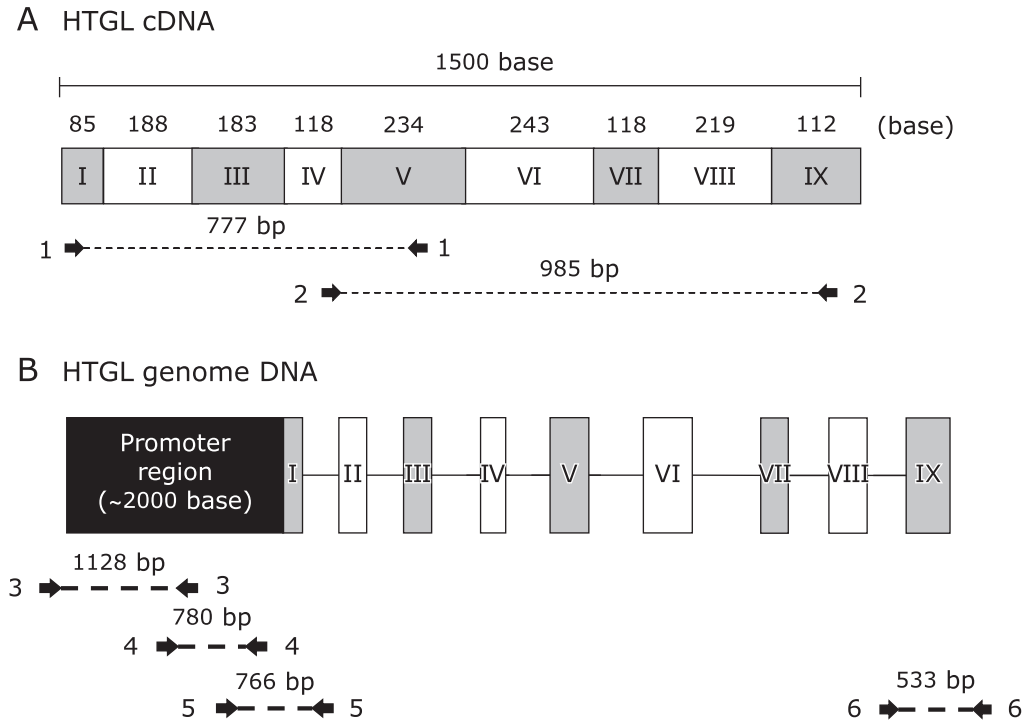

Fig. 7. Strategy for sequence analyses of the HTGL cDNA and 5'-upstream and 3'-untranslated region of the gene in PHT and JW rabbits. cDNAs were synthesized from total RNAs isolated from PHT and JW rabbits. Overlapping HTGL cDNA fragments were amplified by PCR reactions and subjected to sequence analyses (A). The 5'-promoter region (~2,000 bp) and 3'-untranslated region of the HTGL gene were amplified by PCR using genomic DNAs isolated from PHT and JW rabbits and sequenced (B).

doi: 10.3164/jcbn.13-21

This work was supported in part by the Japan Society for the Promotion of Science (JSPS) Strategic Young Researcher Overseas Visits Program for Accelerating Brain Circulation Program.

Abbreviations

CETP	cholesterol ester transfer protein
FAS	fatty acid synthase
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
HTGL	hepatic triglyceride lipase
JW rabbit	Japanese white rabbit
LDLR	LDL receptor
LPL	lipoprotein lipase
MTP	microsomal triglyceride transfer protein
PHT rabbit	postprandial hypertriglyceridemia rabbit
SREBP	sterol regulatory element binding protein
T-CHO	total cholesterol
TG	triglyceride
VLDLR	VLDL receptor

Conflict of Interest

No potential conflicts of interest were disclosed.

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